ACTA CHEMICA SCANDINAVICA 11 (1957) 1745—1751

The Heats of Hydrolysis of Some Alkyl Thiolesters

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The heats of hydrolysis of some alkyl thiolesters have been determined at 25°. For the idealized, isothermal hydrolysis reactions

$$\mathrm{RSAc(l) + H_2O(l) \rightarrow RSH(l) + HOAc(l)}$$

the changes in enthalpy at 25°C were found to be: -0.95 ± 0.06 kcal.mole^{-1} for R = Et; -0.93 ± 0.06 kcal.mole^{-1} for *n*-Pr; -1.09 ± 0.06 kcal.mole^{-1} for *n*-Bu; -1.39 ± 0.07 kcal.mole^{-1} for *i*-Pr; -3.00 ± 0.07 kcal.mole^{1-} for *t*-Bu.

The interest which biochemists show towards S-acyl compounds today is fully evident. The role played by coenzyme A and its acyl derivatives in acyl transfer processes has stressed the importance of studying the energy relationships between different acylated compounds containing O-acyl, N-acyl and S-acyl bonds. The present investigation continues a research program which began with the study of the hydrolysis of thiolacetic acid [1], and aims at a mapping out of the enthalpy changes accompanying different types of trans-acylation processes.

EXPERIMENTAL

Materials

Preparation of the esters. The esters were synthesized in two different ways:

1. Acylation of the corresponding thiol [2]. This method was used to prepare EtSAc from EtSH and Ac_2O, and *t*-BuSAc from *t*-BuSH and AcCl.

2. Reaction between thiolacetic acid and the appropriate alkyl bromide [3]. The reaction was carried out in a 500 ml flask with a reflux condenser. To a solution of 0.5 mole of KOH in 150 ml 96 % ethanol was added 0.5 mole of HSAc. The mixture was cooled to room temperature and 0.5 mole of alkyl bromide was slowly added. After a few minutes the solution became warm and KBr precipitated. After one hour the mixture was poured into 2 l of water. The oil was separated and the water phase extracted twice with ether. The oil and the extract were combined, and washed with 2.5 N H_2SO_4, saturated $NaHCO_3$ solution and twice with water. The product was dried overnight with anhydrous Na_2SO_4. After removing the ether, the residue was fractionated. Yield *ca.* 80 %. This method was used to prepare *n*-Bu, *n*-Pr and *i*-Pr thiolacetates. The method is convenient and the use of evil-smelling thiols is avoided. Attempts to prepare *t*-BuSAc by this method failed.

Purity of the esters. The esters were purified by distillation through a 10-plate column. Purities were >99.5 %, as judged by measurements of their equivalent weights. The

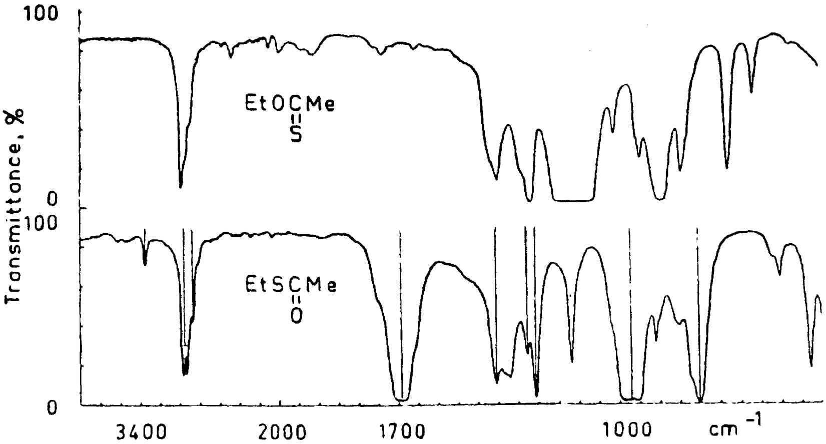

Fig. 1. Infrared spectra of ethyl thionester and ethyl thiolester.

esters showed a neutral reaction, and did not absorb iodine. The equivalent weights were determined after alkaline hydrolysis by titration of both RSH and HOAc.

Determination of RSH. The hydrolysing liquid consisted of an alcoholic NaOH solution (0.5 N NaOH in 50 % ethanol; 10 moles per mole of ester). In this medium the hydrolysis was quantitative within 10 min. However, the thiol formed is very sensitive to air oxidation at high pH values. The reaction was therefore carried out in a nitrogen atmosphere in a long-necked flask with glass stopper. A thinwalled glass ampoule containing 3 mmoles of ester was dropped into the flask and broken by shaking. After about 10 min the solution was cooled to *ca.* −10°C and an excess of glacial acetic acid added. The thiol was titrated with 0.1 N iodine solution in 90 % alcohol. It is well-known that *t*-BuSH cannot be titrated quantitatively with iodine [4].

Determination of HOAc. The hydrolysis was carried out as described above but in a weakly alkaline medium (2 moles NaOH per mole of ester). After leaving overnight the sample was titrated with 0.1 N HCl to pH 7. The thiol was then blown off with CO_2-free air and the solution was titrated to pH 10. Equivalent weight, density and refractive index for the thiolesters synthesized are listed in Table 1.

Table 1. Equivalent weight, density and refractive index for the synthesized thiolesters, RSAc.

R	Equivalent weight			d_4^{25}	n_D^{25}
	RSH	HOAc	Theoretical		
Et	104.6	104.9	104.17	0.9728	1.4568
n-Pr	118.5	118.5	118.19	0.9532	1.4580
n-Bu	132.9	132.9	132.22	0.9390	1.4590
i-Pr	118.1	118.3	118.19	0.9368	1.4510
t-Bu	—	132.3	132.22	0.9207	1.4511

Infrared spectra of the esters. The infrared spectra for the thiolesters have been determined and compared with that of ethyl thionester. It was found that the thiolesters did not contain any detectable amount of thion isomer. Fig. 1 shows the spectra of EtOCSMe and EtSCOMe. The vertical lines indicate the absorption maxima common to all the thiol esters. They are characterized mainly by the C = O stretching bond vibration frequency at 1 700 cm^{-1} and its overtone at 3 400 cm^{-1}.

Auxiliary substances. The thiols used were freshly distilled before their heats of solution were determined. The estimated purity (from determinations of equivalent weights) was better than 99 % except for EtSH which was 98.0 % pure. The density and refractive index for *t*-BuSH was d^{25}_{4} 0.7942; n_D^{25} 1.4205 in good agreement with values from the literature [5]. The acetic acid was purified by freezing out three times. The purity was found to be 99.5 % (titration with NaOH).

Apparatus

The reactions were carried out in an isothermal calorimeter which will be described in detail elsewhere. The system consisted of a steel reaction vessel with a combined stirrer and ampoule holder and a heating element. The vessel was surrounded by a thick-walled copper jacket. The space between the vessel and the jacket was evacuated to 0.1 mm Hg. The temperature change was determined by use of a thermistor (Stantel U 236120).

Calibration

The determination of the heat capacity of the calorimeter was carried out by passing a known current for a known time through the calibration resistance in the calorimeter. The overall error in the calibration measurements was ±0.16 %. The calibrations were carried out on the system after the hydrolysis. Owing to the different behaviour towards oxygen of *t*-BuSH compared with other thiols the slope of the resistance-time curve was different in the two cases. Therefore, the hydrolyses were carried out at slightly different temperatures. In order to minimize errors from small variations in ε with temperature, two series of calibration experiments were performed, namely between the thermistor resistances 2 125 and 2 085 Ω (heat capacity ε_1) and between 2 155 and 2 115 Ω (heat capacity ε_2). Experiments corresponding to reactions III and IV (below) and reactions I and II for R = *t*-Bu were carried out in the latter temperature interval, reactions I and II where R = Et, *n*-Pr, *i*-Pr, *n*-Bu were performed between 2 125 and 2 085 Ω. A check on the accuracy of the heat capacity values was obtained by the determination of the heat of solution of KCl in water. The values obtained agreed within the limits of error with those in the literature.

Procedures

The calorimeter was charged with 90 ml of 0.8 N sodium hydroxide solution in water-ethanol (2 : 3). The sealed glass ampoule contained about 3 mmoles of ester (thiol, glacial acetic acid or water). After equilibration, the reaction was started by breaking the ampoule. The hydrolysis was quantitative within 5 min and the duration of the reaction period was 7 min. Thereafter, a linear time-temperature relationship was established. The short reaction period makes the corrections for heat exchange with the surroundings very small and the initial and final thermistor resistances (R_i and R_f, respectively) could thus be obtained graphically.

The large excess of alkali (*ca.* 25 times) greatly speeds up the hydrolysis reaction and is also favorable from another point of view since the degree of protolysis of the sodium mercaptides formed in the reaction becomes independent of small variations in the amount of ester hydrolyzed. As stated above, the thiols are extremely easily oxidized in an alkaline medium and the heat of oxidation is appreciable. Attempts to free the reaction system completely from oxygen were unsuccessful. Therefore, it was impossible to avoid a false slope of the time-temperature curve during the after period, leading to erroneous values of the heat leakage. Also, the oxidation occurring during the reaction period introduced a considerable error. The same errors, however, occurred in the determinations of the heats of solution of the thiols, and to some extent, these errors cancelled out in the final results. In order to ascertain that no errors should be introduced from variations in oxygen content of the calorimeter liquid, and also to ensure complete cancellation in the final results, 4 mmoles of the appropriate thiol were added prior to beginning the experiment. Thus, the oxidation occurred also in the initial period. The limiting factor on the rate of oxidation was the availability of oxygen as the thermal leakage values obtained were identical with those obtained in experiments without thiol present.

Corrections to standard states

The ideal isothermal hydrolysis reaction V is obtained from eqns. I to IV, corresponding to the reactions taking place under actual experimental conditions.

I	$RSAc(l) + H_2O(l) = RSH(soln) + HOAc(soln)$	ΔH_1
II	$RSH(l) = RSH(soln)$	ΔH_2
III	$HOAc(l) = HOAc(soln)$	ΔH_3
IV	$H_2O(l) = H_2O(soln)$	ΔH_4
V	$RSAc(l) + H_2O(l) = RSH(l) + HOAc(l)$ $\Delta H^\circ = \Delta H_1 - \Delta H_2 - \Delta H_3 + \Delta H_4$	ΔH°

Units of measurement

The results of the calorimetry experiments are expressed in terms of the defined calorie equal to 4.1840 abs. joules and refer to the isothermal process at 25° and to the true mass. The molecular weights were computed from the 1951 table of international atomic weights [6].

RESULTS

The experimental results are summarized in Tables 2 to 5. Table 2 gives two series of calibration experiments, one performed in the interval 2 125 to 2 085 Ω, the second between 2 155 and 2 115 Ω (ε_1 and ε_2, respectively). The first column gives the input of electrical energy, the second the expression proportional to the temperature increase and the third the value of the heat capacity in calories per unit in $\log(R_i/R_f)$.

Table 2. Calibration experiments.

q, cal.	$\log(R_i/R_f) \times 10^4$		$E \times 10^{-2}$
48.59	82.25		59.08
48.45	82.29		58.87
48.26	81.77		59.02
49.48	84.19		58.77
		Mean	58.93 ± 0.06
48.11	82.07		58.62
47.62	81.53		58.41
48.69	83.37		58.40
48.21	82.31		58.57
		Mean	58.50 ± 0.06

In Table 3 the first column gives R in RSAc, the second column the amount of ester hydrolyzed (corrected to 100 % purity). The fourth column gives the amount of heat evolved during the experiment and the last column ΔH_1 in kcal.mole^{-1}.

Table 3. Determinations of heats of hydrolysis of a number of alkyl thiolesters, RSAc.

R	mmoles	$\log(R_i/R_t) \times 10^4$	q, cal	$-\Delta H$ kcal.mole^{-1}
Et	3.230	94.85	55.84	17.29
	3.278	96.09	56.57	17.26
	3.245	95.03	55.95	17.24
	3.154	92.27	54.32	17.22
			Mean	17.25±0.02
n-Pr	2.813	80.20	47.22	16.79
	2.870	81.64	48.07	16.75
	2.946	83.37	49.09	16.66
	2.936	83.40	49.10	16.72
			Mean	16.73±0.02
n-Bu	2.931	81.91	48.27	16.47
	3.106	86.92	51.22	16.49
	2.987	83.59	49.26	16.59
	3.010	84.60	49.85	16.56
			Mean	16.50±0.02
i-Pr	3.293	97.51	57.41	17.43
	3.096	92.36	54.38	17.56
	2.925	87.04	51.25	17.52
	2.958	87.86	51.73	17.49
			Mean	17.50±0.02
t-Bu	3.086	98.95	57.88	18.76
	3.136	100.08	58.54	18.67
	3.167	101.09	59.14	18.67
	3.106	99.05	57.94	18.65
			Mean	18.69±0.02

Table 4 contains the pertinent heats of solution measurements.

Table 5 contains a summary of the observed data and the heat of hydrolysis of the idealized reaction V.

DISCUSSION

In biochemical literature the thiolesters are often spoken of as being "energy-rich" [7,8]. This expression is meant to imply that a large amount of standard free energy, $\Delta F°$, is released when the S-acyl bond is broken *i.e.* by hydrolysis. Quantitatively, it is sometimes stated that, if $-\Delta F°$ exceeds 8 kcal.*, the compound contains an "energy-rich" bond.

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* See for example Fruton, J.S. and Simmonds, S. *General Biochemistry*, John Wiley & Sons, Inc., New York 1954, p. 355.

Table 4. Heats of solution measurements.

Substance	mmoles	$\log(R_i/R_f) \times 10^4$	q, cal	$-\Delta H$ kcal.mole^{-1}
EtSH	3.503	30.38	17.74	5.06
	3.596	31.15	18.19	5.06
	3.588	30.90	18.05	5.03
			Mean	5.05±0.02
n-PrSH	3.215	25.40	14.84	4.62
	3.024	23.34	13.64	4.51
	3.192	24.77	14.47	4.53
			Mean	4.55±0.02
n-BuSH	3.007	21.53	12.58	4.18
	3.249	23.16	13.53	4.16
	3.154	22.36	13.07	4.14
			Mean	4.16±0.02
i-PrSH	3.047	25.40	14.84	4.87
	2.930	24.12	14.09	4.81
	3.345	28.08	16.40	4.90
			Mean	4.86±0.02
t-BuSH	2.852	21.68	12.67	4.44
	3.058	23.31	13.63	4.46
	3.306	25.01	14.62	4.42
			Mean	4.44±0.02
HOAc	3.298	63.79	37.55	11.39
	2.836	55.37	32.59	11.49
	3.020	58.84	34.63	11.47
	3.473	68.07	40.07	11.54
			Mean	11.47±0.03
H_2O	3.168	1.17	0.69	0.22
	2.934	1.10	0.64	0.22
			Mean	0.22±0.02

The equation relating a change in standard free energy, $\Delta F°$, to changes in standard entropy, $\Delta S°$, and standard enthalpy, $\Delta H°$, is

$$\Delta F° = \Delta H° - T\Delta S°$$

Table 5. Summary of experimental data and values of heats of hydrolysis of the idealized reaction $RSAc(l) + H_2O(l) = RSH(l) + HOAc(l)$.

R	$-\Delta H_1$	$-\Delta H_2$	$-\Delta H_3$	$-\Delta H_4$	$-\Delta H°$ at 25°C
Et	17.25	5.05	11.47	0.22	0.95
n-Pr	16.73	4.55	11.47	0.22	0.93
n-Bu	16.50	4.16	11.47	0.22	1.09
i-Pr	17.50	4.86	11.47	0.22	1.39
t-Bu	18.69	4.44	11.47	0.22	3.00

A large ΔF° value may be traced back either to a large ΔH°-term or to a large $T\Delta S^\circ$-term or to both terms, ΔH° and ΔS° in this case having different signs.

The present investigation shows that the value of $-\Delta H^\circ$ for simple alkyl thiolesters is only from 1 to 3 kcal.mole^{-1}. Therefore, if $-\Delta F^\circ$ is to be large, a large increase in entropy must occur during the hydrolysis.

The close analogy between the hydrolysis of an alkyl ester and an alkyl thiol ester

$$\mathrm{ROAc} + \mathrm{H_2O} \rightleftharpoons \mathrm{ROH} + \mathrm{HOAc}$$
$$\mathrm{RSAc} + \mathrm{H_2O} \rightleftharpoons \mathrm{RSH} + \mathrm{HOAc}$$

makes it unlikely that the hydrolysis of the S-acyl compounds should be accompanied by a large change in ΔS°. It is known that ΔH° and ΔF° for the hydrolysis of an O-ester have nearly the same values, both being *ca.* 0—2 kcal.mole^{-1}. The change in entropy is therefore small. From known values of entropies of alcohols and thiols on one hand, and non-hydrogen bonding compounds like ethers on the other, it is likely that the $T\Delta S^\circ$-term for the hydrolysis of a thiolester is *ca.* 1—1.5 kcal.mole^{-1}. It is therefore very probable that simple alkyl thiolesters are not "energy-rich" in the sense referred to above.

Acknowledgement. The author wishes to thank Professor B. Bak and co-workers, University of Copenhagen, for determining the infrared spectra. This investigation has been supported by the *Swedish Natural Science Research Council* and the *State Council of Technical Research.*

REFERENCES

1. Sunner, S. and Wadsö, I. *Trans. Faraday Soc.* **52** (1956) 1088.
2. Rylander, P. N. and Tarbell, D. S. *J. Am. Chem. Soc.* **72** (1950) 3021.
3. Brinzinger, H., Pfannenstill, K. and Kling, K.-E. *Chem. Ber.* **83** (1950) 87.
4. Barltrop, J. A., Hayes, P. M. and Calvin, M. *J. Am. Chem. Soc.* **76** (1954) 4359.
5. Mathias, S. *J. Am. Chem. Soc.* **72** (1950) 1897.
6. Wichers, E. *J. Am. Chem. Soc.* **74** (1952) 2447.
7. Wieland, Th. and Bokelmann, E. *Ann.* **576** (1952) 20.
8. Lynen, F. *Angew. Chem.* **67** (1955) 463.

Received July 31, 1957.